Arch. Hydrobiol.	72	1	133—138	Stuttgart, Juni 1973

A culture system for *Artemia*, *Daphnia*, and other invertebrates, with continuous separation of the larvae

By P. Sorgeloos* and G. Persoone, Ghent

With 1 figure in the text

Abstract

An easily constructed apparatus is described in which adult invertebrates can be reared with automatic feeding and with automatic and continuous separation of the offspring from the adults.

Introduction

In the opening address to the 1970 Helgoländer Symposium on cultivation of marine organisms, and its importance for marine biology, Kinne (1970) very pertinently pointed out that culturing nowadays constitutes a serious bottle-neck in the advancement of marine sciences.

As we emphasized in a previous paper (Sorgeloos & Persoone, 1972) one of the main reasons for this situation undoubtedly is the lack of suitable culture apparatus.

For experimental research it is highly desirable to have at one's disposal a flow-through culturing system for adult organisms which is kept under controlled environmental conditions and equipped with an automatic and continuous device for separating the offspring. By harvesting the larvae daily one can start at will various experiments, either fundamental ones (feeding, growth rate, etc...) or bio-assays, with juveniles of exactly the same age.

In most of the classic static-jar systems either the larvae or the adults are individually removed from the culture after liberation of the progeny.

Dewey & Parker (1964) who reviewed the literature pertinent to *Daphnia* culture, concluded that "... little attention has been given to the problem of rearing large continuous populations of known age".

They developed a "culture gallon with separator" for semi-automatic separation of the larvae of *Daphnia magna* from the adults. The adults are

---

* "Aspirant", at the Belgian National Foundation for Scientific Research (N. F. W. O.).

cultured in a funnel-shaped chamber with a fine-mesh-screen bottom, connected with a removable collecting bottle. The separation is based on the positive phototactic behaviour of the larvae, which migrate into the collecting bottle after illumination.

Removal of the bottle, containing the larvae always results in a spill of water during separation. Moreover air bubbles under the plankton gauze must be removed with a pipette. This system requires one man-hour daily for operation and maintenance.

With these inconveniences in mind, we developed a more automatic culturing and separation unit which we use both for crustaceans such as *Artemia* and *Daphnia* and for a variety of other pelagic invertebrates.

Description of the apparatus

The apparatus consists essentially of four perspex transparent cylinders with funnel-shaped bottoms (A, B, C, D), a water collector (E) as described by Sorgeloos & Persoone (1972) and a food air-water lift system with terminal collecting vial and overflow (F). Figure 1 shows a lateral view of the apparatus. The funnel-shaped bottom of cylinder A (algal-culturing chamber) has a lateral connection for air-bubbling and a waste-drain stop-cock.

Air-water lift system 1 & 2 (polyethylene tubing) is connected with the food-collecting vial 3 which possesses an overflow 4. Siphon 5 extends to a certain depth into vial 3 and is connected to siphon 6 of the water collector E. Cylinder B (adult culturing chamber) is preferentially mounted on a screw jack stand, to permit minor adjustments of height.

The funnel part is separated from the cylinder by a fine mesh-screen glued into the system with a non-toxic resin. Communicating tube 8 connects cylinder B to the vertical tube 9 into cylinder C.

Vessel B can be emptied through a lateral drain 7, entering the cylinder just above the screen. Cylinder C (larval-collecting chamber) is provided with a waste-drain stop-cock 10 and a lateral drain 11 for collecting the larvae. Tube 12 connects cylinders C and D, and is provided with a small planktongauze-cylinder inside vessel C.

Cylinder D (water supply for water collector E) has an overflow 13, slightly below the level of tube 12 and its lower part is connected by a long polyethylene tube 14 with the air-water lift 15. Air-bubbling is provided by a small aquarium air-pump which is switched on for 5 minutes every half hour by an electric clock (we used the Microflash type nr. SYA 8021, "La Vedette" Paris — France).

The dimensions of the apparatus which we normally use are:

Cylinder A: diameter: 15 cm
heigth: 140 cm
water volume (approximately): 20 l

Cylinder B: diameter: 10 cm
heigth: 30 cm
water volume (approximately): 1.5 l

Cylinder C: diameter: 30 cm
heigth: 20 cm
water volume (approximately): 8 l

Cylinder D: diameter: 7 cm
heigth: 20 cm
water volume (approximately): 5 l

Watercollector E: diameter: 3.5 cm
heigth: 45 cm
water volume (approximately): 0.5 l.

A. algal culturing chamber.
B. adult culturing chamber with drain stop-cock (7) and communicating tubes (8 & 9) to cylinder C.
C. larval collecting chamber with waste-drain stop-cock (10) and lateral drain (11) for collecting the larvae.
D. water supply cylinder with overflow (13). cylinder D is connected to water collector E by communicating tube (14) and air-water lift (15).
E. water collector with siphon (6).
F. food-air-water lift system (1 & 2) with terminal collecting vial (3), overflow (4) and siphon (5).

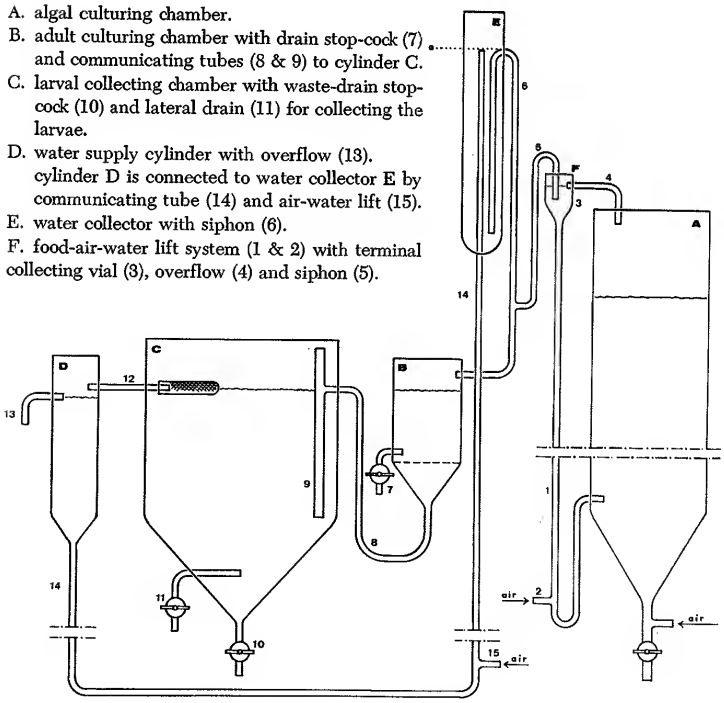

Fig. 1. Schematic presentation of the apparatus.

Working principle

Cylinder A is filled with the liquid medium (either fresh water or sea water) containing the necessary food particles.

Since food for many invertebrates consists of algae, the vessel should be illuminated by a vertical light tube. The food is kept in suspension by vigorous aeration, which provides the necessary carbon dioxide for the algae.

Air-water lift 2 ensures a continuous supply of food suspension to vial 3, with an overflow back to the food chamber. Cylinders B, C and D are filled with water to the level of the overflow 13.

The flasks B and C communicate by tube 8 and the water level in both cylinders will be the same.

The adults are brought into cylinder B and the system is ready for operation.

Every half hour the small air-pump is automatically switched on (for 5 minutes only) and lifts water (from cylinder D) to the water collector E. At level "a", siphon 6 starts operating and the well-oxygenated water flows into cylinder B. This flow down of the water through the tube creates an underpressure in siphon 5, which after a short time starts to empty vial 3. Thus each half hour a small and constant volume of food is brought to the adult organisms and their progeny. If larvae are present, they normally swimm around in the cylinder B among the adults, and some of them already pass through the bottom screen into the juvenile chamber C.

The inflow of a rather large volume of water into vessel B results in a transfer of the same volume of medium from cylinder B to C (along tube 8) since the water level in those communicating chambers will tend to equilibrate.

Most of the larvae cannot resist this current and are gently carried along from the adult chamber to the juvenile compartment. Since each half hour an analogous shift of larvae and water from B to C occurs, the whole offspring of one female, passes in a few hours into the juvenile vessel. Since the larvae even in cylinder C receive a sufficient amount of food, the system is completely automatic and self-sustaining. One can harvest at will (for mass culturing) juveniles of different ages through stop-cock 11. After emptying vessel C completely and refilling it with fresh medium, one can collect the off-spring after one day, all the individuals being 24 hours old at most.

Application and Discussion

The apparatus has been used extensively in our laboratory for culturing *Artemia salina* and *Daphnia magna* fed a preserved food or living

algae. Experiments in progress clearly indicate that its use can be extended to various other pelagic invertebrates.

Concerning the culture of *Daphnia magna,* several authors mention difficulties in finding a synthetic freshwater medium in which reproduction of this common crustacean is good (FREEMAN, 1963; BOYD, 1957; DEWEY and PARKE, 1964).

In our experiments the formula for soft freshwater precognized by CAIRNS (1969) always gave excellent results for continuous culturing of *Daphnia.* The algal food which we used and on which the latter animals thrive very well was either *Chlorella pyrenoidosa* or *Scenedesmus opoliensis.*

For *Artemia* we used the artificial seawater formula precognized by DIETRICH and KALLE (1963) and, as food, the algae *Dunaliella sp., Phaeodactylum tricornutum* and *Monochrysis lutheri* as well as commercial brands of dried algae. Mass-culturing of the algae was done separately following a routine technique which will be described elsewhere. At the end of the logarithmic growth phase, the algae were harvested, centrifuged, resuspended in either sea water or fresh water and introduced into the algal chamber, of which the contents are sufficient for at least one week. The density of the algae in the algal chamber has to be chosen carefully (after dilution with the water of the water-collecting tube) in order to obtain an adequate concentration of food particles in the adult-culturing chamber.

For *Daphnia* a food concentration in the latter vessel of 10^5 cells per ml, which means about 10^6 cells per ml in the algal chamber is necessary.

The number of adult organisms was kept at 25 per liter, which appears to be the optimal density for reproduction (ADEMA, personal communication).

In these conditions, and at a temperature of approximately 25° C, each adult produces every other day approximately 40 larvae. The apparatus has to be kept in continuous light if one wants to avoid production of males. Finally the *Daphnia* in the adult-culturing vessel are best changed every third week.

Though we have not yet found the optimal food-concentration for, nor the optimal density of adults of *Artemia,* we obtain a daily yield of several hundreds of larvae from 100 adults (male/female ratio $^1/_2$) and a food concentration of approximately 2×10^5 algal cells per ml.

Having used the present apparatus for more then six months, we have not yet been faced with special problems.

Summary

An easily constructed apparatus is described in which adult *Artemia, Daphnia* and other pelagic invertebrates can be reared with automatic feeding and

with automatic and continuous separation of the offspring from the adults. Essentially it consists of cylinders in transparent non-toxic material with funnel-shaped bottom, connected by polyethylene tubing. Culturing methods are given for *Daphnia magna* and *Artemia salina.*

Résumé

Un dispositif, simple à construire, est décrit, pour la culture d'Artemia, de Daphnies et d'autres invertébrés pélagiques, avec séparation automatique et continue de la progéniture. Il est fabriqué essentiellement avec des cylindres en matériel transparent et non toxique, dont les parties basales en forme d'entonnoir communiquent au moyen de tuyaux souples en polyéthylène. Des méthodes de culture sont décrites pour *Artemia salina* et *Daphnia magna.*

References

Boyd, J. E. (1957): The use of *Daphnia magna* in the microbioassay of insecticides. — Ph. D. Thesis. Pennsylvania State Univ. 205 pp.

Cairns, J., jr. (1969): Fish bioassays — Reproducibility and rating. — Revta. Biol. 7: 7—12.

Dewey, J. E., & Parker, B. L. (1964): Mass rearing of *Daphnia magna* for insecticide bioassay. — J. econ. Entomol. 57: 821—825.

Dietrich, G., & Kalle, K. (1963): General Oceanography. — Interscience, New York.

Freeman, L. (1953): A standardized method for determining the toxicity of pure compounds to fish. — Sewage Ind. Wastes 25: 845—848.

Kinne, O. (1970): International Symposium "Cultivation of marine organisms and its importance for marine biology". Opening address. — Helgoländer wiss. Meeresunters. 20: 1—5.

Sorgeloos, P., & Persoone, G. (1972): Three simple culture devices for aquatic invertebrates and fish larvae with continuous recirculation of the medium. — Mar. Biol. 15: 251—254.

Walne, P. R. (1956): Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. — Fish. Invest., London. Ser. 2, 20: 23 pp.

Address of the authors:

P. Sorgeloos & Prof. Dr. G. Persoone, State University of Ghent, Interfacultair Centrum voor de studie van Lucht-, Bodem- en Waterverontreiniging, K. L. Ledeganckstraat 35, B-9000 Ghent (Belgium).